SYMPOSIUM ON BIOCHEMICAL BASES OF MORPHOGENESIS IN FUNGI[1]

IV. Molecular Bases of Form in Yeasts

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Determinant of Cellular Form in Yeasts

The shape of a yeast cell is determined by the form of its rigid cell wall. Walls isolated from yeast cells retain the characteristic appearance of the yeast from which they were obtained. Studies on the chemical composition of cell walls have provided information on the nature of the macromolecular components of the wall fabric. In some instances, electron micrographs of wall material reveal the spatial arrangement of certain of the macromolecular aggregates. How physical properties associated with a given macromolecular configuration confer dimensional stability on the wall tapestry into which the macromolecular component is woven poses questions that intrude upon many domains of science.

In this paper, the fractionation of isolated cell walls and the separation of macromolecular components of the wall will be considered. Chemical analyses and physical characterization of the wall components will be examined. Modes of linkage among the several wall components (many of which are water-soluble) to provide a discrete, rigid form will be discussed. Finally, some consideration will be given to cell-wall modifications that accompany cellular growth and cellular division.

Isolation of Cell Walls of Yeasts

"In the study of cell walls of yeasts and other micro-organisms, chemically intact, clean cell wall material is a first requirement" (30). To achieve this succinctly stated aim is not easy. A cell population, as nearly homogeneous as possible, of a culture, the identity of which is known with some certainty, is obtained under repeatable conditions. The cells, washed free from components of the culture medium, are either (i) ruptured mechanically by collision impact, shear, or some transduced means such as sonic oscillation, or (ii) exposed to enzymatic action that causes cellular lysis. In either case, cell-wall material must be separable from other cellular components for subsequent analyses to be meaningful.

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Mechanical Rupture of Viable Cells

Isolation of cell-wall fragments from yeasts ruptured by agitation with glass beads in a Mickle disintegrator was reported by Houwink, Kreger, and Roelofsen (21) and by Northcote and Horne (45). The material thus obtained contained no whole cells, was practically free from cellular debris, and appeared in an electron microscope to comprise the outer membranes of the cell. Essentially similar approaches, employing various methods of agitating cell pastes with glass beads, were employed by Falcone and Nickerson (9), Eddy (8), Kessler and Nickerson (27), Korn and Northcote (29), and Crook and Johnston (7).

Despite the excellence of the Hughes press (22) for rupturing yeast cells to obtain cell-free enzyme preparations, it has not proved possible (in studies conducted in our laboratory) to separate intracellular particulate elements from the slightly "torn" cell. In the operation of this press, the shear exerted by ice crystals on the cell mass acts as an ultrafine "knife" that produces fine cuts in the cell wall. Other methods, such as high-pressure extrusion (13), are available for rupturing yeast cells, but are employed principally for obtaining cell-free enzyme preparations, and do not appear to have been used to obtain cell-wall preparations.

Rupture of Yeast Cells by Sonic Oscillation

Whereas cell rupture by sonic oscillation has proved of value in isolating cell walls from many types of bacteria, the walls of many yeasts are too sturdy to be ruptured by this means. Certain apiculate yeasts, however, have been found to possess relatively delicate cell walls, and to be ruptured easily in a 10-kc magnetostriction oscillator; almost complete breakage of cells is obtained within a reasonable time (34). The relative weakness of cell walls of these yeasts is seen from the fact that 50% of the cells of *Hanseniaspora uvarum* and of *Saccharomycopsis guttulata* were ruptured within 6 min by treatment in a 10-kc oscillator, whereas 32 min were required to obtain comparable rupture of *Saccharomyces cerevisiae*.

Enzymatic Digestion of Walls of Intact Cells

Experiences of several workers with enzyme preparations from the snail (*Helix pomatia*) were reviewed by Svihla, Schlenk, and Dainko (59). These authors reported that incorporation of a sulfur-containing amino acid in the culture medium of *Candida* (*Torulopsis*) *utilis* increased susceptibility of cell walls to digestion by *Helix* enzyme preparations. The spheroplasts so obtained were markedly more stable than had been reported for spheroplasts of *S. cerevisiae* by other workers. An enzyme preparation obtained from a species of *Streptomyces*, isolated by Salton's (53) procedure, has been reported to lyse cell walls of various yeasts, and to be effective in transforming intact cells into spheroplasts (15). Curiously, a culture labeled *C. utilis* was reported to be very susceptible to lysis by this enzyme preparation, whereas *Torula* [*sic*] *utilis* was not.

Criteria of Purity

Inherent in the use of any of the procedures involving mechanical rupture of cells to obtain clean preparations of cell-wall fragments is an elaborate routine of centrifugation and washing with water, sucrose solutions, and buffers. The course of purification of the preparation is commonly followed by light microscopy, including phase-contrast and dark-field examination of wet-mount and stained preparations. So far as is known, all yeasts are intensely gram-positive, whereas even slightly ruptured cells are gram-negative. As the preparation becomes increasingly free from contamination with small particulate elements, it is examined in an electron microscope to verify freedom from such contamination (see Fig. 1). The criteria of purity thus include: absence of intact, stainable cells; absence of small, spherical bodies; microscopic homogeneity; and constancy of analyses for nitrogen.

FRACTIONATION OF ISOLATED CELL WALLS

After the development of methods for preparing clean cell-wall preparations from yeasts, procedures were developed for fractionation of cell walls and separation of their components. By a mild treatment with weak alkali at 25 C, Falcone and Nickerson (9) solubilized a portion of the yeast cell wall. The material solubilized was shown to be a mannan-protein complex; the properties of this component of the cell wall will be discussed in detail in a following section. This initial work led to recognition of the polysaccharide-protein nature of wall components,

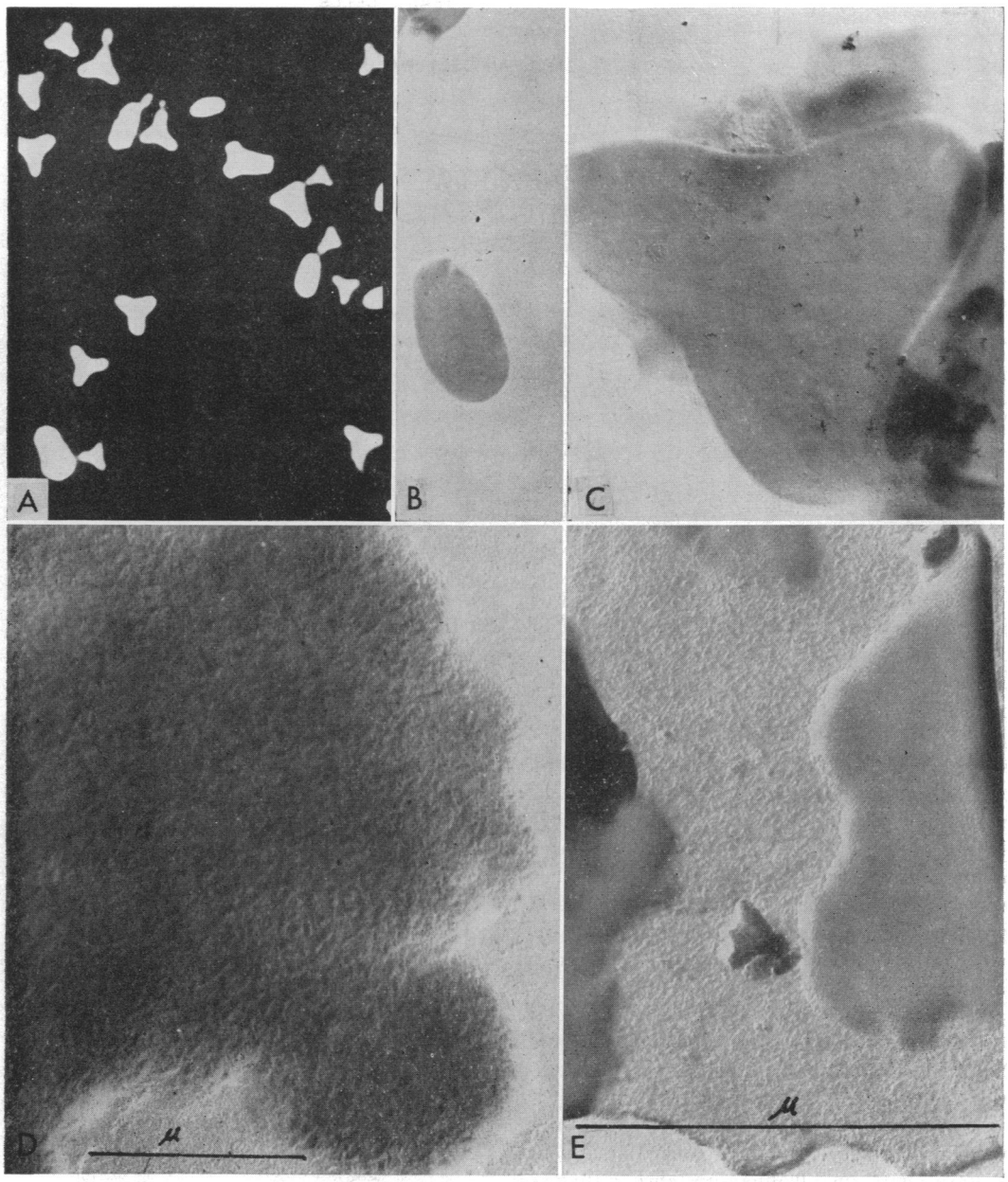

FIG. 1. *Electron micrographs of isolated cell walls of various yeasts. Note correspondence between cell-wall outlines (B and C) and cellular shapes (A) for ellipsoidal and triangular forms of Trigonopsis variabilis. Clean cell walls employed in analytical studies: Candida albicans (D) and baker's yeast (E).*

and pointed to the necessity for removal of lipids as an initial step in the fractionation procedure. Subsequent work by Kessler and Nickerson (27) followed the scheme of fractionation outlined in Fig. 2. Clean cell-wall preparations were rendered free from lipids, by a three-stage extraction procedure, then suspended in 1 N KOH for 1 hr at 25 C under an atmosphere of nitrogen. This treatment leads to cleavage of ester linkages, as will be detailed under con-

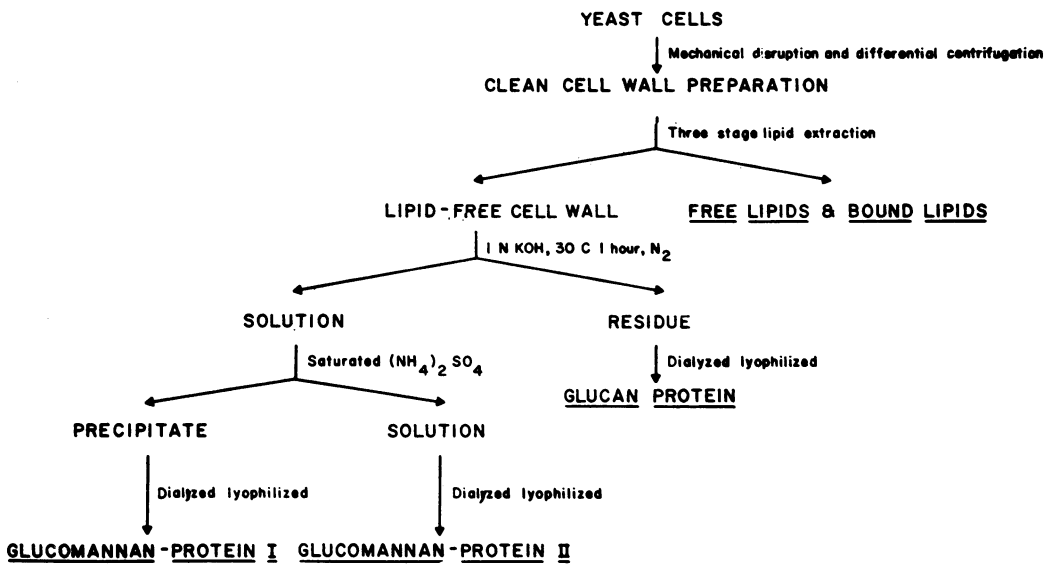

FIG. 2. *Outline of procedure of Kessler and Nickerson (27) for fractionation of yeast cell walls.*

siderations of modes of linkage among wall components. It is known that glycosidic linkages are stable to aqueous alkali at 25 C under nitrogen (66). Material solubilized by this procedure was treated with ammonium sulfate, yielding both a soluble and an insoluble polysaccharide-protein fraction. These fractions, together with the fraction not solubilized by the mild alkali treatment, were subjected to thorough dialysis against running water, were concentrated at low temperature to small volumes, and then were lyophilized. The fractions were obtained as white powders. Physical and chemical analyses on these wall components are presented in a following section.

A fractionation procedure somewhat similar to that just outlined was described by Korn and Northcote (29). These authors also isolated cell-wall components in three fractions. Their procedure (shown in Fig. 3) did not entail prior removal of lipids, but the prolonged treatment (3 days) with an alkaline substance (ethylene diamine) may be presumed to be comparable to the mild alkaline treatment depicted in Fig. 2. Fraction A of Korn and Northcote is a mannan-protein, closely similar to the mannan-proteins isolated by Falcone and Nickerson (9) and by Eddy (8), as compared in Table 1. Fraction C of Korn and Northcote is a glucan protein that is probably comparable with that isolated by Kessler and Nickerson (27). The glucomannan-protein isolated by Korn and Northcote (their fraction B) most likely combines some of the properties of the two glucomannan-proteins obtained by the fractionation procedure of Kessler and Nickerson.

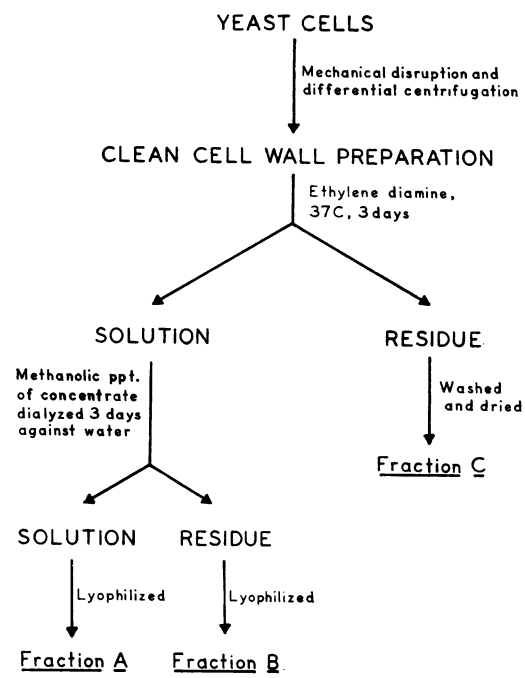

FIG. 3. *Outline of procedure of Korn and Northcote (29) for fractionation of yeast cell walls.*

TABLE 1. *Properties of mannan-protein isolated from purified yeast cell walls*

Organism	Protein-mannan ratio	Sedimentation constant
*Saccharomyces cerevisiae**	1:12	4.3×10^{-13}
S. carlsbergensis†	1:12	4.5×10^{-13}
S. cerevisiae‡	1:9	4.2×10^{-13}

* Data of Falcone and Nickerson (9).
† Data of Eddy (8).
‡ Data of Korn and Northcote (29).

Enzymatic Digestion of Isolated Cell Walls

Preparation of a "cell-wall medium" for selective isolation of microorganisms capable of hydrolyzing cell-wall components was described by Salton (53). Isolated cell walls of *Candida* (*Torulopsis*) *pulcherrima* were dispersed in a washed agar-mineral salts medium. From a soil inoculum, several strains of *Streptomyces* were obtained that produced a clearing of the opaque medium as a result of lytic action on the cell-wall dispersion. Cultures of myxobacteria, including chitin-decomposing *Cytophaga johnsonae* and two strains of *Myxococcus fulvus*, also effected extensive lysis of yeast cell-wall material. Employing Salton's procedure, Phaff et al. (47) isolated a species of *Bacillus* which produced clearing of media containing a dispersion of cell walls from baker's yeast. Concentrated cell-free culture fluid digested yeast cell walls optimally at pH 6.5; glucose and two oligosaccharides were detected among the products of digestion. The enzymatic activity of this preparation appeared to be directed against the glucan component of the cell wall. The enzyme preparation did not seem to affect intact yeast cells; viable vegetative cells were not transformed into protoplasts except in the case of the yeastlike organism *Ashbya*.

Eddy (8) treated isolated cell walls of strains of *S. carlsbergensis*, *S. cerevisiae*, and *S. uvarum* with enzyme preparations from snail and malt, and with crystalline trypsin and papain. With the snail enzyme, isolated walls dissolved completely, and both glucose and *N*-acetylglucosamine were detected among the products of hydrolysis. By the use of papain, about 50% of the weight of isolated walls was solubilized into nondialyzable hydrolytic products. All of the phosphate of the wall was thus solubilized, and was recovered in a phosphomannan-protein complex. Eddy stated that the nondialyzable polysaccharide released by treatment of isolated cell walls of *S. carlsbergensis* with crystalline papain at pH 7.6 contained relatively equal amounts of glucose and mannose although, initially, mannose residues were solubilized almost exclusively. After digestion of walls for 10 min with papain, a substance was solubilized that was precipitable with copper (Fehling's solution) and contained about 2% nitrogen. The substance recovered as the copper complex thus contained protein-polysaccharide in a ratio of 1:12 and exhibited a sedimentation constant of 4.5 ($\times 10^{-13}$ cgs) units. Aside from precipitability with Fehling's solution, this substance bears considerable similarity to the mannan-protein isolated by Falcone and Nickerson (9) from purified cell walls of *S. cerevisiae* from which lipid had not been extracted (see Table 1).

Unquestionably, selective degradation of isolated cell walls by enzymatic means possesses inherent advantages over even the mildest of the alkaline hydrolysis procedures now available. Hydrolytic activity of enzyme preparations from *Helix* is commonly attributed to the presence of β-glucanase. Complete digestion of isolated walls by such preparations (8) emphasizes the fundamental role of the glucan component in maintaining integrity of the cell wall. Recently, the action of highly purified proteolytic enzymes (obtained from species of *Streptomyces*) on isolated cell walls has been examined in this laboratory. In Fig. 4 is shown the rate of lysis of cell walls isolated from strain 18.29 of *S. cerevisiae* by keratinase (produce by *S. fradiae*) and by pronase (produced by *S. griseus*). Lysis of cell walls by keratinase under these conditions was complete within 42 min, and within 72 hr with pronase. In contrast to the action of keratinase and pronase on walls of this strain of *S. cerevisiae*, markedly less effect was noted on walls of *C. albicans* incubated under similar conditions. The contribution of protein to integrity of cell walls of the high-protein strain of *S. cerevisiae* is evident as a result of cell-wall lysis by these powerful proteolytic enzymes. On the other hand, the protein components of *C. albicans* cell walls are less susceptible to proteolytic attack, and another component, presumably glucan, must bear the principal role in maintaining structural integrity. In this light, one might surmise that isolated cell walls

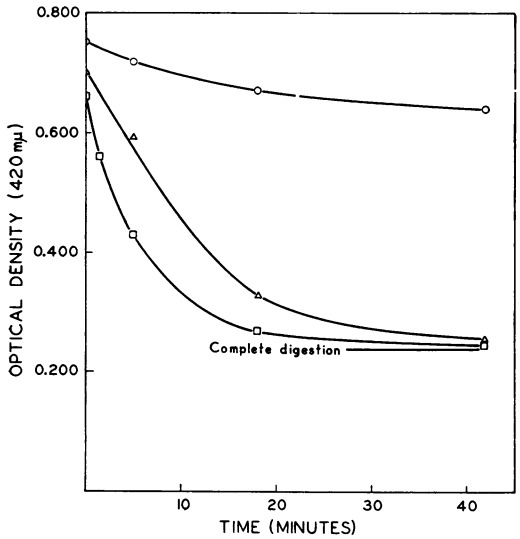

FIG. 4. *Enzymatic lysis of suspension of isolated cell walls of Saccharomyces cerevisiae strain 18.29 by pronase (○), keratinase conjugate (△), and crystalline keratinase (□). Optical density equivalent to complete digestion is indicated. Tris(hydroxymethyl)aminomethane (tris) buffer, pH 7.0, employed for pronase; tris buffer, pH 9.0, with both keratinase preparations; 10 mg of cell walls exposed to 100 μg of enzyme in 5-ml reaction volume at 25 C.*

of yeasts known to be rich in lipid, especially phospholipid, but deficient or lacking in glucan, i.e., *Trigonopsis variabilis*, might be lysed preferentially by a lipase or lipoprotein lipase, rather than by β-glucanase or proteolytic enzyme.

Macromolecular Components Isolated From Cell Walls

As emphasized in the preceding section, procedures employed for the fractionation of isolated cell walls serve to yield polysaccharide-protein complexes in a high state of purity and in a form little different from that in which they exist *in situ* in the cell wall. The procedures are not designed, however, to yield lipids in the macromolecular combinations in which they undoubtedly exist in the cell wall.

Lipids

In several investigations, lipids have been liberated from clean cell-wall material by a three-stage extraction procedure involving in sequence: (i) treatment with ethanol-ether at 25 C, (ii) extraction with chloroform at 25 C, and (iii) treatment at 50 C with ethanol-ether containing 1% HCl. The extractions are usually carried out in the dark, under nitrogen atmosphere, with freshly distilled solvents. This procedure was developed by Anderson and colleagues for fractionation of the lipids of *Mycobacterium tuberculosis*. An extensive description of the method was given by Peck (46), and a concise survey of the materials isolated from *M. tuberculosis* by this procedure was presented by Stodola (58). Material extracted by ethanol-ether and by chloroform at 25 C is commonly termed "free lipid," but a better name would be "readily extractable lipid" since such materials from *M. tuberculosis* have been found to include quite complex lipopolysaccharides and lipopeptides. Material resisting extraction by neutral solvents, but obtained on treatment with acidic ethanol-ether, is usually termed "bound lipid" and, in some instances (*see* 58), has been found to comprise lipopolysaccharide. In view of the substantial amounts of lipid associated with cell walls of some species of yeasts (see Table 2), it is surprising that no lipid-containing macromolecular component has yet been isolated from yeast cell walls.

The contribution of macromolecular lipid-containing entities to cellular configuration can only be conjectured at the present time. Studies by Hurst (23) on the orientation of lipids in yeast cell walls have shown that lipid components

TABLE 2. *Lipid content of purified cell walls*

Organism	Free lipid I	Free lipid II	Bound lipids	Total lipids
Baker's yeast*	0.81	0.42	6.88	8.11
Saccharomyces cerevisiae 18.29*	8.43	0.94	0.82	10.19
Candida albicans 582*	0.20	0.32	0.57	1.09
C. albicans 806*	0.51	0.56	0.03	1.10
C. albicans RM806*	0.59	0.43	0.16	1.18
S. carlsbergensis†	0.27	0.14	0.15	0.56
S. pastorianus†	0.16	0.31	0.55	1.02
Trigonopsis variabilis‡				
Ellipsoidal form	0.60	0.12	4.7	5.42
Triangular form	1.9	0.08	6.7	8.68

* Data of Kessler and Nickerson (27).

† Data of Klaushofer, Mähr, and Szilvinyi (28).

‡ Data of SentheShanmuganathan and Nickerson (54).

probably contribute to cellular architecture. This contribution of lipids is not widely appreciated, and the basis thereof remains to be established. As shown in Fig. 5, a dry yeast cell is not flattened but remains a prolate spheriod, even though thoroughly dehydrated. If this dry cell is treated with certain lipid solvents, such as chloroform, ethanol-ether, or butanol, marked flattening occurs (23). Electron-diffraction studies on cells flattened in this manner show that lipids remaining in the cell are oriented so that their long axis is normal to a plane tangent to the cell. Although circumstantial evidence of this type definitely implicates a role for lipid components in the framework of cellular architecture, it remains for future studies to provide evidence as to whether lipids serve as bricks or mortar, and to indicate whether lipids are bound to protein or polysaccharide moieties of the wall fabric.

Polysaccharide-Protein Complexes

Since the first isolation of a polysaccharide-protein complex from yeast cell walls (9), the widespread occurrence of such macromolecular complexes has been demonstrated (8, 27, 29, 28, 7). Properties of mannan-proteins obtained from isolated yeast cell walls that *had not been defatted* are shown in Table 1. The data reported from the different laboratories are in excellent agreement. With respect to precipitability of a mannan-protein complex by Fehling's solution, it must be recognized that the basis of blue-colored complex formation between mannan and copper is not understood; as first pointed out by Cifonelli and Smith (6) in the case of invertase, a mannan-protein, formation of a colored complex between mannan and copper may be suppressed by the union between mannan and protein.

Furthermore, precipitation of a polysaccharide by copper *does not constitute evidence that it is composed solely of mannose residues;* the heteropolymeric glucomannan (mannose-glucose, 2:1) studied by Rebers and Smith (48) was readily precipitable by Fehling's solution and was thus separated from accompanying alkali-soluble polyglucosan. The glucomannan of *S. cerevisiae* (mannose-glucose, 1:1) studied by von Euler and Fodor (64) also was isolated and purified by formation of its copper complex.

As we have just seen, precipitability of a polysaccharide as a blue copper complex on reaction with Fehling's solution does not guarantee that the substance is composed of mannose residues alone. On the other hand, it is clear that some alkali-soluble mannose-rich polysaccharides isolated from yeast cell walls do not react in a characteristic manner with Fehling's solution (i.e., formation of a blue polysaccharide-copper precipitate, stable to washing with dilute alkali, but soluble in dilute acid). For example, the

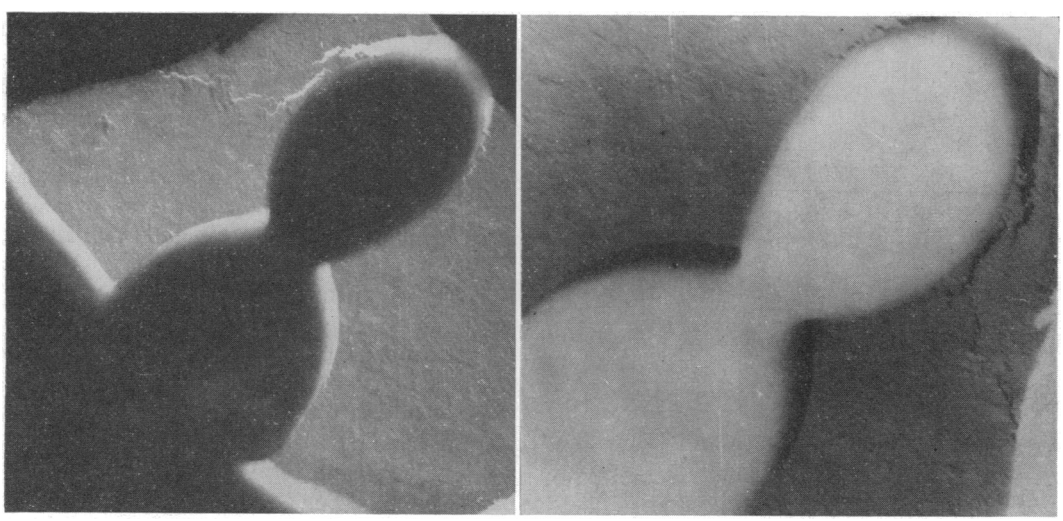

FIG. 5. *Electron micrograph of intact, dehydrated cell of Candida albicans. Preparation shadowed with chromium, and examined in Trüb Täuber 40-kv electron microscope; magnification, 4,850 ×; prints from positive and negative films show dehydrated cell is not flattened.*

mannose-containing polysaccharide extracted from cell walls of the triangular form of *T. variabilis* gives a bluish-white precipitate that dissolves on subsequent washing with dilute alkali (54). Shifrine and Phaff (55) also reported on an "atypical" mannose-containing material obtained from cell walls of *S. guttulata*.

Reports of absence of mannan in *Sporobolomyces roseus*, as stated by Kreger (30), and in *Rhodotorula* sp. (16, 30) are questioned by Crook and Johnston (7) on the basis of their finding (Table 3) that isolated cell walls of *Sporobolomyces roseus* and *R. aurantica* are rich in mannose-containing polysaccharide. The resolution of this apparent contradiction must await study of the mannose-containing polymer after it has been isolated from cell walls of *R. aurantica*. It may well prove to be a "mannan" that does not form an alkali-soluble copper complex. Whatever the outcome of this question may be, it is certain that the material will be markedly different from the glucomannans of *S. cerevisiae* and of *C. albicans* for, as Kemp (26) has shown (and as will be discussed in detail farther on in this paper), these substances are responsible for serological cross reactions between *S. cerevisiae* and *C. albicans*. It is highly relevant to this discussion, therefore, to note that Tsuchiya et al. (61), in an extensive serological investigation of a large number of yeasts, could find no cross-reaction among *R. glutinosa*, *R. minuta*, *Cryptococcus neoformans*, and *C. albicans*. Thus, these yeasts possess no antigen in common, and, therefore, four serological groups of yeasts were defined by these studies. (Of approximately 180 recognized species of yeasts, 67 have been studied to date by Tsuchiya and coworkers.) Thus, it will be of much interest to learn whether mannose-containing polymers are present in both the *R. minuta* and *R. glutinosa* groups. With considerable confidence, one may again venture the opinion that if the answer to this question indicates the presence of mannose-containing polymers in cell walls of both species of *Rhodotorula* then these polymers will be markedly different from each other.

TABLE 3. *Sugars present in acid hydrolysates of isolated yeast cell walls**

Organism	Glucose	Mannose	Galactose	Fucose	Glucosamine
Saccharomyces cerevisiae	4+	3+	—	—	TR
Candida utilis	5+	2+	—	—	TR
Sporobolomyces roseus	2+	4+	+	+	3+
Rhodotorula aurantica	+	5+	+	+	+
Schizosaccharomyces octosporus	6+	TR	—	—	—
Nematospora gossypii	6+	+	—	—	—

* Data of Crook and Johnston (7); relative abundance (+), trace (TR), or absence (—) indicated.

The fact that mannan-protein is most readily isolated from cell walls that have not been freed of lipid raises questions pertinent to our consideration of linkages among cell-wall components; this matter will be treated subsequently. Although there is a tendency to consider the so-called "mannan" or "yeast gum" isolated from intact yeast cells by chemical extraction to be pure and to be composed only of mannose residues, the evidence for this assumption is not at all clear. Earlier work on isolation of polysaccharide components from intact cells has been reviewed in some detail elsewhere (42). Suffice it to say at this time that investigations by von Euler and Fodor (64), in which the use of hot, strong alkali was avoided, revealed "yeast gum" to be a glucomannan with a ratio of mannose-glucose of 1:1.

It is of some interest, therefore, to note that pioneer studies on yeast wall components did reveal the presence of glucose in fractions that were presumed to be mannan. Roelofsen (51) reported that after treatment of a lipid-free preparation of yeast cell walls with 2% NaOH at 100 C for 15 min the presence in solution of both mannose and glucose was detected. Roelofsen pointed out that a 30-min treatment with hot 2% NaOH apparently dissolves all of the mannan and in addition some of the "glucan," a finding in accordance with that of Houwink and Kreger (20).

It must be recognized that the isolation of a glucomannan-containing material does not necessitate that it be a heteropolymer of glucose and mannose. The water-soluble glucomannan-proteins (GMP) isolated from yeast cell walls by mild procedures may be a type of copolymer, comprising a polyglucose and a polymannose linked to the protein component of the complex, or some at least of the glucose and mannose may be linked together in a heteropolymer from which

an oligosaccharide containing both glucose and mannose may be isolated. The occurrence of glucomannan heteropolymers has been demonstrated in extracts of a variety of plant materials. Iles mannan, isolated from a plant tuber, was shown to be a mixture of two polysaccharides, a glucomannan and a polyglucosan, in the ratio of about 6:1 (48). Based on the isolation of mannisidoglucose and glucosidomannose as products of acetolysis, the glucomannan was proved to be a heteropolymer (57).

The relative proportions of the three polysaccharide-protein components isolated from yeast cell walls by the procedure of Kessler and Nickerson (27) are shown in Table 4. The data reported for *S. carlsbergensis* and *S. pastorianus* were obtained by Klaushofer, Mähr, and Szilvinyi (28). Relative proportions of mannose and glucose in each of the fractions isolated from seven strains of yeast are shown in Table 5. Traces of mannose remaining in glucan protein were also noted by Korn and Northcote (29) in the material (fraction C) corresponding to glucan protein that is isolated by their procedure. A ratio for mannose-glucose of 1:1 has been found for most samples of GMP-I that have been isolated, and a ratio of 2:1 for GMP-II.

TABLE 4. *Polysaccharide-protein complexes of yeast cell walls*

Organism	Glucan-protein	GMP-I	GMP-II
Baker's yeast*	41.6	13.6	34.7
Saccharomyces cerevisiae 18.29*	28.3	55.8	11.9
Candida albicans 582*	47.4	3.0	27.2
C. albicans 806*	45.0	2.1	34.3
S. carlsbergensis†	12.1	32.2	23.1
S. pastorianus†	9.6	37.7	25.9

* Data of Kessler and Nickerson (27).
† Data of Klaushofer, Mähr, and Szilvinyi (28).

Physical analyses conducted on the polysaccharide-protein fractions isolated from yeast cell walls include: electron microscopy, ultracentrifugation, and moving-boundary electrophoresis. Electrophoretic mobilities determined for GMP-I isolated from high-protein strain 18.29 of *S. cerevisiae* are presented in Table 6. As shown in Fig. 6, preparation GMP-I from *S. cerevisiae* 18.29 is essentially homogeneous in its main component; there is present in this material a small amount of relatively uncharged material. Most polysaccharides that are uncombined with charged groupings appear homogeneous, but have little or no migration in an electrophoretic system; however, the same cannot be said of the polysaccharide-protein complexes isolated from yeast cell walls by the procedure outlined in Fig. 2 or 3. These complexes bear considerable charge owing to the attached protein, and electrophoretic analysis of their mobility and homogeneity is meaningful. Although preparations of GMP-I are not dialyzable and, thus, of particle weight in excess of about 10,000, the weight of this material is too low to permit characterization of homogeneity by ultracentrifugation (42).

In contrast, GMP-II clearly shows a major peak by ultracentrifugal analysis that has a sedimentation constant S = 3.3; on prolonged centrifugation, the presence of a small amount of lower particle weight material is revealed (Fig. 7).

TABLE 5. *Relative content of glucose and mannose in cell-wall components*

Organism	Glucan-protein		GMP-I		GMP-II	
	Glucose	Mannose	Glucose	Mannose	Glucose	Mannose
Baker's yeast*	95	5	47	53	35	65
Saccharomyces cerevisiae 18.29*	95	5	45	55	33	67
Candida albicans 582*	98	2	54	46	36	64
C. albicans 806*	95	5	48	52	37	63
C. albicans RM806*	97	3	52	48	29	71
S. carlsbergensis†	93	7	60	40	—‡	—
S. pastorianus†	97	3	44	56	—	—

* Data of Kessler and Nickerson (27).
† Data of Klaushofer, Mähr, and Szilvinyi (28).
‡ Not determined; material employed in immunochemical studies.

Since the first isolation of glucan from yeast by Salkowski in 1894, the extraordinary insolubility of this material and its resistance to alkaline hydrolysis have been widely noted. In the procedure of Bell and Northcote (2) for isolation of glucan from baker's yeast, intact cells are treated with hot 6% NaOH; glucan is recovered, after a washing procedure, from material undissolved by this treatment. In the procedures of Trevelyan and Harrison (60) and Chung and Nickerson (5) for fractionation of yeast polysaccharides, glucan is obtained as the residue remaining after treatment with 30% KOH at 100 C for 15 to 30 min. By these procedures, glucan has been found to comprise about 10% of the cellular dry weight of baker's yeast or about 30% of the dry weight of the yeast cell wall.

In an attempt to account for the remarkable insolubility of glucan, Northcote (44) examined the sorption of water vapor by glucan to obtain information on the extent of internal hydrogen bonding in this material. The insolubility of cellulose is explainable on the basis of strong internal hydrogen bonding of hydroxyl groups. With yeast glucan, however, almost no internal hydrogen bonding was evident (Table 7), and essentially every hexose residue was accessible for sorption of water vapor. The very low "crystalline-to-amorphous" ratio for glucan is noteworthy, even though the preparation used by Northcote had not been reduced to a disorganized powder but retained structural features of the cell wall. In comparison, the fraction of cellulose fibers inaccessible to water is high (0.68), attributable to the crystalline portion of the fibers, and the crystalline-to-amorphous value is correspondingly high. Northcote concluded, that, "The very insoluble nature of glucan depends therefore on other factors, the most probable being that the molecule of glucan must be much larger than that of cellulose." In view of the fact that Korn and Northcote (29) have recently shown that glucan, prepared by standard methods, contains approximately 6% glucosamine and a variety of amino acid residues, it is quite likely that some measure of the insolubility of the highly branched glucan polymer may b

TABLE 6. *Electrophoretic mobility of glucomannan protein I**

pH	Buffer	Mobility
		$\mu \times 10^5$
2.5	Glycine-HCl	+3.499
8.4	Tris-HCl	−6.657

* Glucomannan protein I from strain 18.29 of *S. cerevisiae*; concentration 0.5% in 0.05 M buffer made 0.15 M in NaCl.

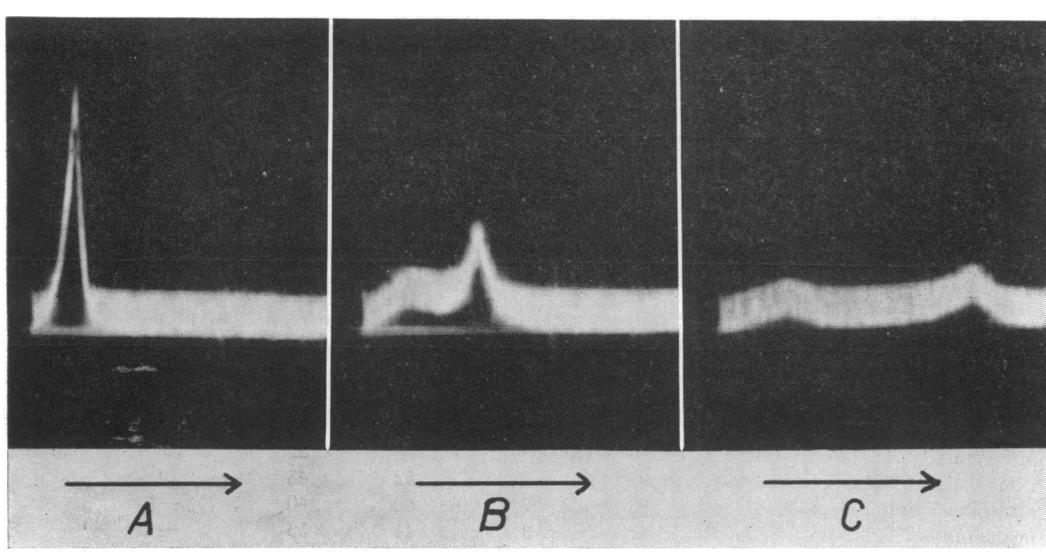

FIG. 6. *Electrophoretic mobility of glucomannan protein I derived from strain 18.29 of Saccharomyces cerevisiae in tris buffer (pH 8.4); conditions as stated in Table 6.*

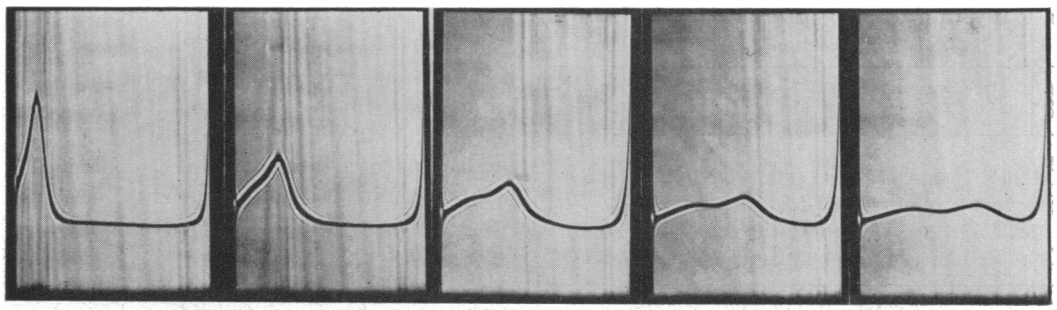

FIG. 7. *Sedimentation of glucomannan protein II in analytical ultracentrifuge at 59,780 rev/min in phosphate buffer (pH 7.0) with NaCl (0.1 ionic strength); photographs, left to right, at 32, 64, 96, 128, and 160 min; major peak sedimentation constant, S = 3.3.*

TABLE 7. *Sorption of water vapor by cell-wall polysaccharides**

Substance	Fraction of monomer units inaccessible to H_2O	Crystalline-amorphous ratio
Mannan	0.44	0.78
Glucan	0.02	0.02

* From Northcote (44).

due to the inclusion of these substituents. Attention will be called in a subsequent section to the difficulty in removing amino acid residues proximal to a polysaccharide moiety.

Cell-Wall Protein

Quantitative analysis of the amino acids of fractions isolated from cell walls of *S. cerevisiae* revealed the protein associated with each fraction to be closely similar in composition (27). As shown in Table 8, the markedly acidic character of these proteins is evident; glutamic and aspartic acids together account for 31% of the total recovery in the case of GMP-I, 35% with glucan protein, and 39% of GMP-II.

In the polysaccharide-protein complexes solubilized from cell walls of baker's yeast by their ethylene diamine procedure, Korn and Northcote (29) found only 2 N-terminal amino acids (alanine and glycine) in fraction A, an indication that this protein may be a specific compound. The same N-terminal amino acids were found in the protein of fraction B, suggesting that the protein might be the same as in fraction A.

Hexosamine-Containing Macromolecules

The necessity for expressing the reservation, implied in the foregoing heading, as to the form in which acetylglucosamine, glucosamine, or other hexosamine is bound in yeast cell walls arises from demonstrations by Kessler and Nickerson (27) and Korn and Northcote (29) that bound hexosamine is present in each of the three fractions separated by their respective procedures (Tables 9 and 10). Not only did Korn and Northcote find glucosamine in acid hydrolysates of the three polysaccharide-protein

TABLE 8. *Amino acid analysis of cell-wall components**

Amino acid	Glucan protein†	GMP-I‡	GMP-II‡
	%	%	%
Glutamic acid	10.9	17.8	9.2
Aspartic acid	29.8	13.1	31.1
Cysteic acid	1.5	0.2	8.4
Tyrosine	2.2	5.0	2.7
Serine	4.1	4.1	4.6
Threonine	6.0	5.0	5.9
Glycine	3.3	3.5	4.6
Alanine	6.4	6.9	6.5
Valine	6.5	4.6	5.4
Proline	7.8	2.2	—
Methionine	—	1.6	—
Isoleucine	4.5	5.1	4.9
Leucine	4.6	9.1	7.0
Lysine	5.1	8.1	6.2
Histidine	—	2.7	—
Arginine	3.7	7.2	3.5
Phenylalanine	3.6	3.8	—

* Data of Kessler and Nickerson (27); values expressed as per cent of total recovery from acid hydrolysates of each component.

† Glucan protein isolated from baker's yeast.

‡ Glucomannan proteins I and II isolated from *Saccharomyces cerevisiae* strain 18.29.

TABLE 9. *Glucosamine content of isolated wall fraction**

Organism	GMP-I	GMP-II	Glucan protein
	%	%	%
Baker's yeast.....	0.34	0.49	0.89
Saccharomyces cerevisiae 18.29.....	0.27	0.79	0.57
Candida albicans 582....	0.91	0.72	2.14

* Data expressed as per cent weight of dry weight of each fraction for hexosamine, determined by analysis in glucomannan protein I (GMP-1), glucomannan protein II (GMP-II), and glucan protein [data of Kessler and Nickerson (27)].

TABLE 10. *Analysis of fractions separated from yeast cell wall by ethylene diamine procedure**

Fraction	Sugar	Nitrogen	Glucosamine	Protein	Phosphorus
		%	%	%	%
A	Mannose	2.0	1.7	11.6	0.30
B	Mannose, glucose	1.0	0.8	5.9	0.12
C	Mannose, glucose	2.7	2.0	15.9	—

* Data of Korn and Northcote (29) for baker's yeast.

fractions isolated from cell walls, but substantial amounts of glucosamine were also present in mannan and glucan prepared from yeast by standard methods. On the basis of the solubility properties of these materials, and the relative ease with which glucosamine could be released therefrom, Korn and Northcote concluded that only a small proportion (ca. 10%) of the total glucosamine in the cell wall could be present in the form of chitin. They conjectured, as did Eddy (8), that glucosamine might serve as a connecting link between protein and polysaccharide components of the complexes.

IMMUNOCHEMICAL PROPERTIES OF WALL COMPONENTS

From rabbits immunized with formalinized cells of *C. albicans*, Jonsen (24) obtained antisera that precipitated water-soluble polysaccharides that had been extracted from *C. albicans* by the formamide procedure of Fuller (14). The precipitins in question were adsorbed by treatment of antisera with intact cells, and Jonsen concluded that these soluble antigens were derived from the yeast cell wall. From the formamide extract of *C. albicans*, Jonsen, Rasch, and Strand (25) isolated a water-soluble polysaccharide that appeared to be electrophoretically homogeneous, albeit of low mobility, and from which both glucose and mannose were obtained on hydrolysis.

The existence of a series of antigens common to species of *Saccharomyces*, *Candida*, and *Torulopsis* has long been appreciated (3, 33, 63, 62, 17). According to the system of Tsuchiya, *C. albicans* and *S. cerevisiae* possess in common thermostable antigens 1, 2, and 3. Kemp (26) repeated and confirmed the antigenic analysis of the species of *Candida* presented by Tsuchiya, and concluded that antigens 1, 2, and 3 are cell-wall components. Using a double agar-gel diffusion technique, Kemp found a line of identity for supernatant soluble antigen of *C. albicans* and GMP-II (either from *C. albicans* or *S. cerevisiae*) when unadsorbed antisera of *C. albicans*, of other species of *Candida*, or of *T. glabrata* were used. In contrast, monospecific antisera containing antibodies for antigens 3 through 9 did not yield a precipitin band with GMP-II. By the same procedure, GMP-I (either from *C. albicans* or *S. cerevisiae*) was found to be reactive with monospecific antiserum 3, but unreactive with monospecific antisera 4 through 9. Thus, the basis for this cross-reactivity rests, in large measure, on the identity or near identity of antigenic components of the respective cell walls.

According to Tsuchiya et al. (61), *C. albicans*, *R. glutinosa*, *R. minuta*, and *C. neoformans* do not show cross-reactions with one another and, therefore, do not possess any antigen in common. It is of interest, thus, to note that Kreger (30) failed to find either glucan or copper-precipitable mannan in *R. glutinosa*. The cell-wall composition of the latter organism must then be quite different from that in *C. albicans* and *S. cerevisiae*.

LINKAGE AMONG COMPONENTS OF YEAST CELL WALLS

In foregoing sections, reference has been made to the manner in which water-soluble polysaccharide-protein entities may be linked to the water-insoluble glucan component of the cell wall, lipid linked to some other wall component,

and polysaccharide linked to protein. Investigations in this difficult area are only in their beginning and, in most instances, must be considered explorative in nature. Among the problems that demand clarification are the nature of lipid binding in the yeast cell wall, and the mode of protein-polysaccharide union.

In view of the ease with which protein-polysaccharide components are solubilized from isolated cell walls on treatment with dilute alkali, one is led to consider the possibility that ester bonds between glucan and the protein of GMP may have been cleaved. In light of the preponderance of acidic amino acids in the protein associated with each polysaccharide fraction, an attempt to discern whether ester bond cleavage occurs during the course of solubilization of wall components was undertaken in this laboratory.

The extent of ester linkage between protein and polysaccharide was estimated by a slightly modified version of the hydroxylamine procedure of Hestrin (19). Purified cell-wall preparations of *S. cerevisiae* were incubated with hydroxylamine (NH_2OH) in varying concentrations of NaOH at 25 C for 60 min. At the end of this time, $FeCl_3$ in acid solution was added. Color, due to iron hydroxamate formed, was estimated spectrophotometrically at 540 mμ, and was expressed with reference to β-pentaacetyl-D-mannose as standard (Table 11). Formation of iron hydroxamate as a result of ester bond rupture was pronounced. The findings indicate that ester linkage occurs between polysaccharide and acidic amino acid residues of a protein moiety. It must be kept in mind, however, that, whereas cleavage of an acetyl-hexose linkage occurs rapidly in a slight excess of alkali, an appreciable exposure to a definite excess of alkali was required to obtain hydroxamate formation with cell-wall preparations.

Further indication that acidic amino acids are bound to polysaccharide in the yeast cell wall is furnished by examination of the products of proteolytic action on isolated cell walls. As mentioned previously, the enzyme keratinase brings about complete dissolution of cell walls of *S. cerevisiae* strain 18.29, and pronase caused substantial digestion of these walls. Chromatographic analysis of both enzyme digests revealed the presence of several free amino acids (Fig. 8), phenylalanine and lysine being most prominent. Glutamic and aspartic acids, although together constituting more than 30% of the total weight of protein in these cell walls, are not evident in these chromatograms. The acidic amino acids remain bound to the polysaccharide components of the cell walls. In their studies on glycopeptides from ovalbumin, in which aspartic acid is linked to carbohydrate, Lee and Montgomery (32) also reported an abnormal resistance to proteolysis for peptide bonds near to carbohydrate residues.

TABLE 11. *Hydroxylamine trapping of alkali-cleaved esterified linkages of protein-polysaccharide*

Previous treatment of cell walls	Ester bond cleavage*		
	0.25 N NaOH	0.75 N NaOH	1.0 N NaOH
	μg	μg	μg
None.....	0	17	70
Urea (6 M).....	0	12.5	50

* Data expressed as μg of iron hydroxamate formed on incubation of 5 mg of cell-wall preparation with hydroxylamine·HCl in stated concentration of NaOH for 60 min at 37 C.

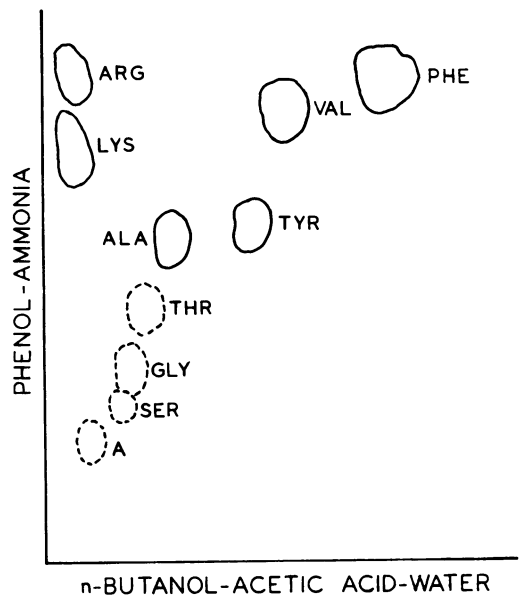

FIG. 8. *Two-dimensional chromatogram of digest of isolated cell walls of Saccharomyces cerevisiae strain 18.29, obtained on treatment with pronase for 60 min at 25 C. Note prominence of phenylalanine and lysine, and almost complete absence of acidic amino acids. Spot A is unidentified; it does not have R_F values for aspartic acid in these solvent systems.*

MECHANISM OF BUDDING IN YEASTS

Cellular multiplication in yeasts occurs chiefly by a process termed budding; thereby, ellipsoidal-

shaped cells give rise to ellipsoidal-shaped buds. In some species of yeasts, the growth process can be uncoupled from the multiplicative process so that elongated, sausage-shaped cells are formed. Many efforts have been devoted toward gaining an understanding of metabolic differences between so-called "normal" yeasts and their "divisionless" form of growth; such studies on species of *Candida* and *Geotrichum* have been reviewed in recent years by Nickerson, Taber, and Falcone (43), Morris (35), and Skinner and Fletcher (56). Among the many results of such studies was the finding that a specific disulfide-reducing process must be maintained in order for the multiplicative (budding) process to ensue. Comparative studies of the metabolism of normal and a divisionless mutant strain of *C. albicans* led to demonstrations of equivalence in growth rates, nutritional requirements, respiratory activity (slightly higher in the divisionless strain), polysaccharide composition, and levels of cystine reductase and glutathione reductase (39, 52, 65). Despite these identities of function in the face of gross morphological differences, the fact emerged that addition of a small amount of cysteine adjacent to the growth of the divisionless strain on solid medium promoted cellular division in this strain. Upon ascertaining that growing cultures of the divisionless strain of *C. albicans* accumulated and reduced tetrazolium dyes massively, whereas cells of the normal strain did not reduce the dye they accumulated (38), it was possible to specify that some major hydrogen-transfer system was blocked in the mutant form. Clearly, this hydrogen acceptor was not oxygen (65). With the isolation of polysaccharide-protein complexes from yeast cell walls, and demonstration of the disulfide content therein (9), it was shortly thereafter found that disulfide links in the GMP are cleaved enzymatically by a protein disulfide reductase contained in mitochondrial particulates derived from normal yeast strains. This enzyme activity is almost entirely lacking in mitochondrial particulates derived from divisionless strains (40, 41). Protein disulfide reductase has since been shown to be of widespread occurrence (18), and to be active with many protein substrates. In view of the fact that both normal and divisionless strains of *C. albicans* possessed active cystine and glutathione reductases (40), one might assume that these enzymes are not responsible for reduction of

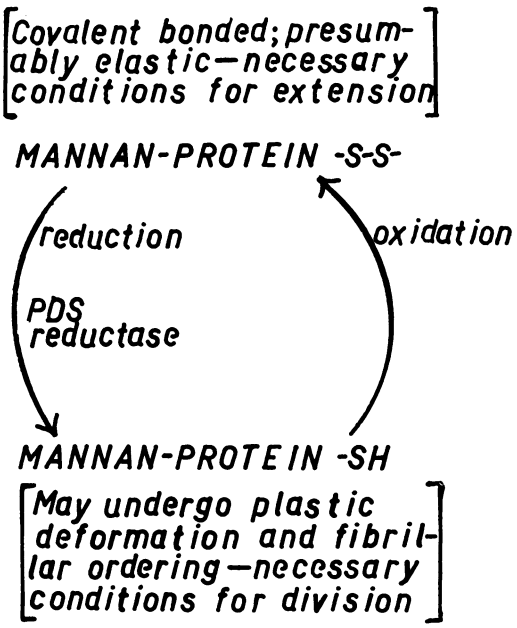

FIG. 9. *Outline of role of protein disulfide (PDS) reductase in chain of events resulting in cellular division of yeasts. From Nickerson and Falcone (41).*

disulfide groups in protein. That this is indeed the case has been shown by Hatch and Turner (18) in studies with purified protein disulfide reductase. Participation of pyridine nucleotides in the operation of protein disulfide reductase has been demonstrated (18), and the enzyme may possess a vicinal dithiol grouping at its active site. Operation of protein disulfide (PDS) reductase in the multiplicative (budding) process of yeasts may be illustrated diagramatically, as shown in Fig. 9.

Localization of SH^- at Site of Budding in Yeasts

Using sectioned cells and a stripping film technique of autoradiography, Robson (49) found that *Eremothecium ashbyii* (grown for 2 days in $S^{35}O_4$-containing medium) concentrated sulfur in cell walls. Growth at this stage was marked by the appearance of bud initials along the length of hyphal elements. In later studies (50), in which tritiated phenyl merucuric chloride was employed to localize SH^- groups via radioautography, a very strong SH^- reaction was detected in hyphal walls at the site of bud

formation (Fig. 10). Autoradiographs of *C. albicans* also revealed SH^- to be localized in cell walls of yeast cells; in cultures of *C. albicans* grown on potato agar, SH^- was detected in walls of yeast cells but not of the filamentous cells that developed on this medium. Robson and Stockley (50) concluded, "Nickerson and Falcone's expectation that SH^- compounds in the region of bud formation could be demonstrated with a suitable cytological reagent has been realized in these experiments."

Physical Aspects of Cellular Division

To understand how enzymatic rupture of covalent bonds among macromolecular components of a cell-wall fabric initiates the process of cellular division, it is necessary to consider certain of the physical properties of cross-linked cell-wall polymers. We may first examine the manner in which a discretely localized, highly plastic area of the cell wall may be established.

Time-lapse cinematography (dark-field microscopy) reveals that a bud is initiated explosively, as a "blow-out" from the mother cell (41, 10). A naked, spherical bud-initial is violently extruded through a gaping hole in a thick cell wall (Fig. 11). In contrast to "synthetic" protoplasts, which persist as naked protoplasmic spheres, the bud-initial, "spheroplast of natural origin," is quickly covered with wall substance, the formation of which commences from the wall of the mother cell at the base of the bud. Thus, formation of wall fabric necessitates the "primer" action of pre-existing wall fabric and, in a special sense, wall fabric constitutes a self-duplicating entity, characteristic of the cell in question. To account for this sequence of events, we may postulate that an enzymatically induced, localized area of increased plasticity was established in the cell wall which failed to withstand the internal fluid pressure; in consequence, the wall ruptured at this "thin spot," and a spherical protoplasmic mass was extruded. In rubber, another covalently bonded polymeric system, increased plasticity results from treatment that leads to rupture of covalent bonding, and the polymeric fabric becomes more readily deformable (11). In their studies on budding of yeasts, Mortimer and Johnson (36) observed, "That the surface area of the cells increases with repeated budding suggests that the bud scar does not replace a portion of the original wall but instead fills a region that has been opened in the cell wall during bud formation." Opening of the cell wall during bud formation is clearly shown in Fig. 11.

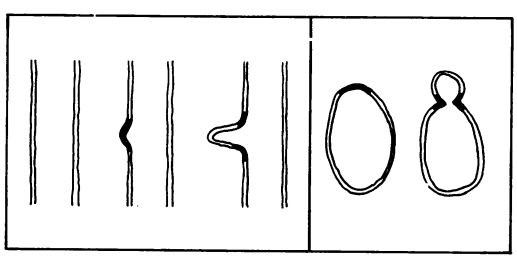

FIG. 10. *Diagrams based on radioautographic localization of SH^- with tritiated phenyl mercuric chloride in: (left) Eremothecium ashbyii and (right) Candida albicans. Note localization of SH^- at site of bud formation. From radioautographs of Robson and Stockley (50).*

That a small, circumscribed portion of the cell wall should become a "target," upon which the plasticizing action of protein disulfide reductase is directed, requires an examination of the "ballistic system" employed by the cell. The location of a site of bud formation is not random. The long axis of a bud is always perpendicula

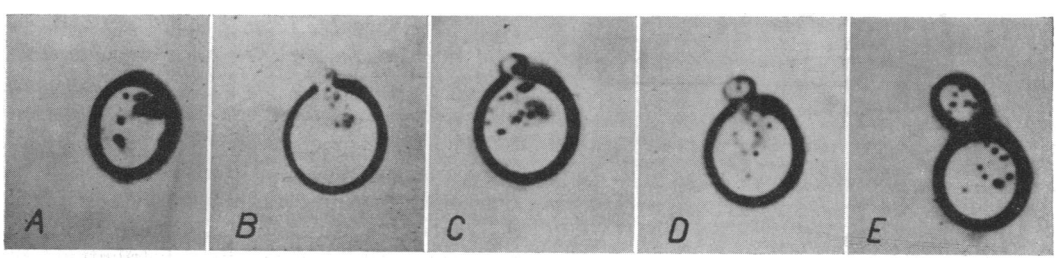

FIG. 11. *Process of budding in Saccharomyces cerevisiae as shown in time-lapse photomicrographs. Note explosive extrusion of naked protoplasmic sphere in B, taken 30 sec after A. Subsequent stages of formation of wall on the bud-initial are shown in C and D; nearly mature bud in E.*

to a plane tangent to the mother cell at the point of contact, as shown in Fig. 12 (37), and a bud arises from a cell at the point of maximal curvature of the cell. Barton (1) observed a sequence in the pattern of budding sites in yeast that may be characterized as a progression through permitted loci of maximal curvature: (i) the distal apex of the prolate spheroid (the pole opposite that marked by the "birth scar" of the cell); (ii) one or more sites in an annulus below the distal apex; (iii) sites in an annulus adjacent to the proximal apex; (iv) lateral sites of budding (detectable) after the cell wall had been marked and deformed by a series of bud scars). Mortimer and Johnson (36) found that a yeast cell budded, on the average, 23.9 times. Confirmation of Barton's findings has been reported by Freifelder (12) from time-lapse photographic studies of multiplying yeasts. Regularity in the position of buds produced successively by a single mother cell was noted (see Fig. 13). The position of the first bud was invariant, but dependent on ploidy, arising from the end opposite the birth scar in polyploid cells. Although no satisfactory explanation could be advanced for the difference in budding pattern between haploid and polyploid cells, the mode of budding is not a genetic character, and, as long as a cell had not budded more than three or four times, one could predict with certainty where the next bud would arise.

What is the physical significance of the geometrical relationships just described? One knows that a fluid in motion exerts greatest force against the wall of its container in the region of maximal curvature of the container. However, selection of a point in a "permitted" annulus of maximal curvature, as a site against which plasticizing action is directed, necessitates that the plasticizing action be localized in discrete particles of "point" size. The fact that protein disulfide reductase is confined to mitochondrial particulates of yeast agrees with theoretical limitations on the confines of the plasticizing agent. It is of interest, then, to note that from a study of rates of budding in strains of *S. cerevisiae* of varying ploidy, Burns (4) concluded that the *rate-limiting process* in cellular division of yeasts is controlled by specific nongenetic units, and that the distribution of these units during division of a cell determines the relative division times of the two resultant cells. Obviously, distribution of mitochondrial particulates (containing protein disulfide reductase) between mother cell and bud satisfies these specifications.

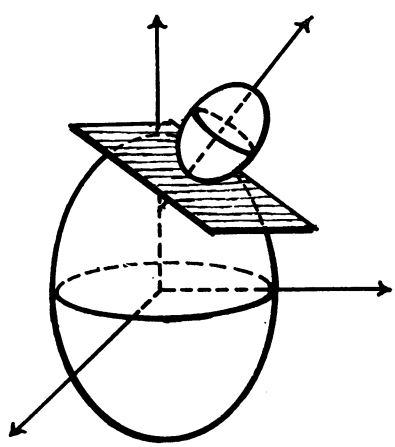

FIG. 12. *Representation of geometrical relationship of bud to mother cell. Long axis of bud is perpendicular to a plane tangent to the mother cell at the point of emergence of the bud.*

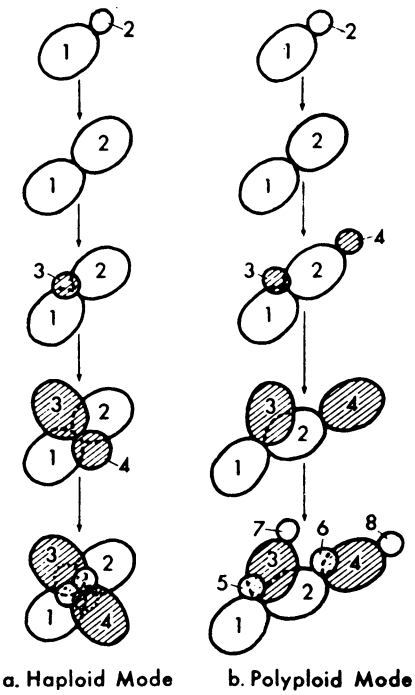

FIG. 13. *Schematic diagram of haploid and polyploid modes of budding in Saccharomyces cerevisiae. From Freifelder (12).*

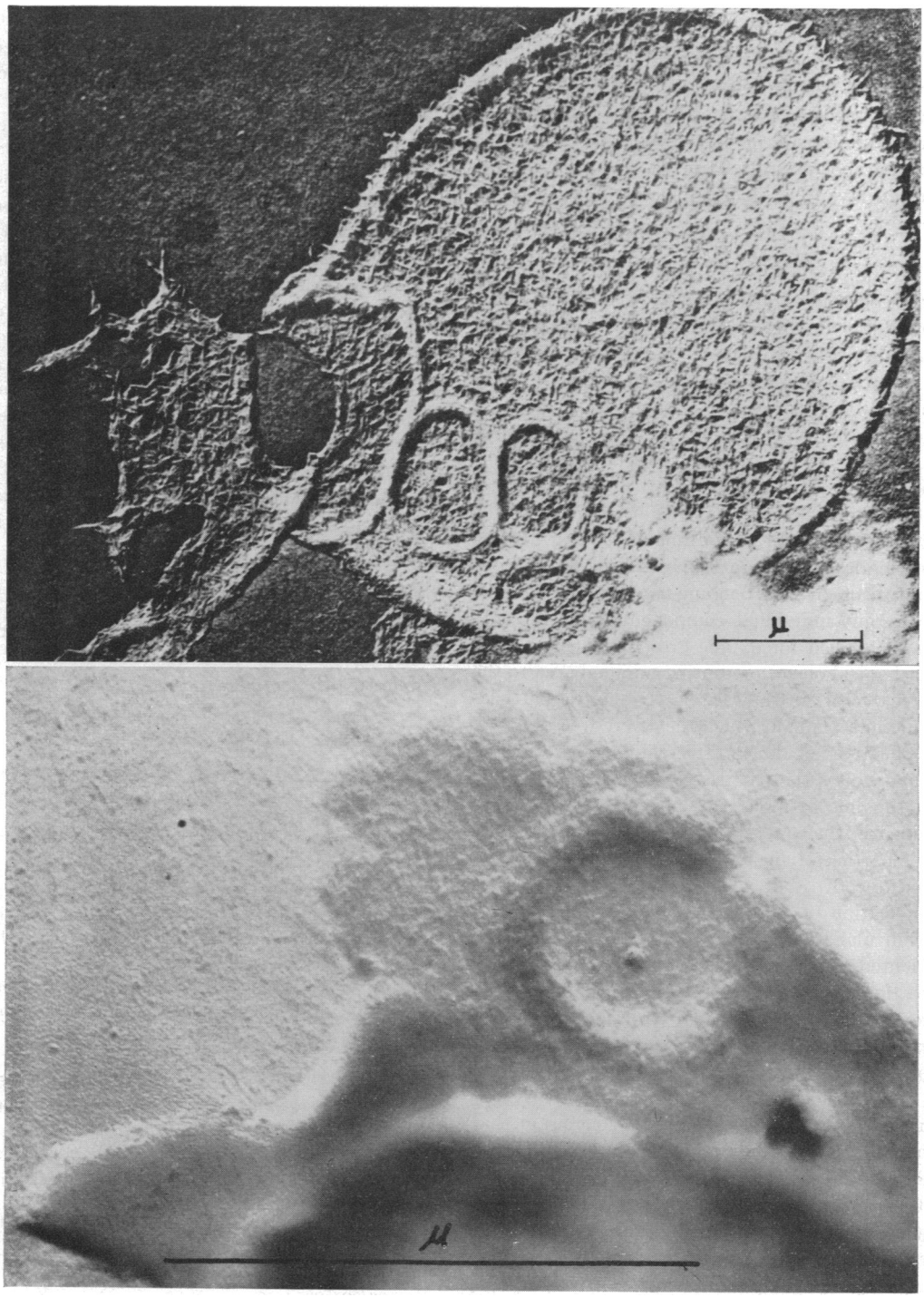

FIG. 14. *Cellular outline of Candida tropicalis (top) revealed as random meshwork of glucan (hydroglucan) fibrils except for ordered arrangement in bud scars [from Houwink and Kreger (20)]. Isolated cell wall of baker's yeast (bottom) showing bud scar as seen from interior of cell [Nickerson et al. (42)].*

Fibrillar Orientation in Bud Scars

A third physical aspect of the division process that will be examined is the orientation of macromolecular components of the cell wall that arises at the site of bud development. Electron micrographs of isolated yeast cell walls, or the glucan layer thereof (Fig. 1 and 14) reveal that polymer formation in the expanding prolate spheroid occurs as a densely intermeshed fibrillar network. As mentioned previously, studies on other polymeric systems (11) reveal that covalent bonding among components increases the modulus of elasticity of the system; maximal covalent bonding demands maximal disorder among the fibrillar components. Except in regions in which budding has occurred, this condition has substantially been achieved in the yeast wall, as seen in the highly random distribution of fibrils in the wall. As a corollary of the statement just made, ordering of components of a polymeric system into a pattern necessitates rupture of covalent bonding to an extent sufficient to permit individual components to "respond" to an applied force. The "response" is a purely mechanical one of presenting minimal area to the force vector. In the case of asymmetric particles lying in a plane in which they were free to move, application of linear stretching force in this plane causes the particles to be oriented parallel to the line of force; this phenomenon is exemplified in the preparation of Polaroid films (31). For particles lying in a plane in which they are free to move, but which they cannot leave, the application of a linear force in a direction perpendicular to the plane causes the particles to be oriented circularly in the plane and tangential to the force vector. This situation is beautifully exemplified in the circular ordering of fibrillar components of the yeast wall at sites of bud formation (Fig. 14).

Thus, evidence from several different experimental approaches supports the conclusion that the process of cellular division in yeasts is the result of a chain of events that begins with the utilization of metabolically generated hydrogen by a specific enzymatic system (protein disulfide reductase) for the reduction of disulfide bonds in polysaccharide-protein complexes of the yeast cell wall. The enzyme is contained in discrete particles within the cell; these particles are in motion, and are seen in time-lapse films to "bombard" sites on the wall. Selection of a site against which this action is directed, to a greater than random extent, is governed, apparently, by purely physical limitations. The consequences that ensue on reduction of disulfide linkages in components of the cell-wall fabric are physical consequences of localized softening of the wall of a container holding fluid under pressure: explosion extrusion of a sphere, and orientation of "loose" fibrils in the plane of the wall tangent to the direction of the explosive force. (The orientation of particles in three-dimensional space is the summation of stress and shearing vectors that combine to produce a circular pattern if stress is exerted normal to the plane of reference, or an elliptical pattern if the stress vector is at other than a right angle to the plane.) After these events, there returns an enzymatic phase, concerned with elaboration of wall fabric on the extruded protoplasmic sphere and the myriad processes involved in elaboration of substance by the growing bud.

ACKNOWLEDGEMENT

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LITERATURE CITED

1. BARTON, A. A. 1950. Some aspects of cell division in *Saccharomyces cerevisiae*. J. Gen. Microbiol. **4**:84–86.
2. BELL, D. J., AND D. H. NORTHCOTE. 1950. The structure of a cell-wall polysaccharide of baker's yeast. J. Chem. Soc., p. 1944–1947.
3. BENHAM, R. 1935. Cryptococci—their identification by morphology and serology. J. Infect. Diseases **57**:255–274.
4. BURNS, V. W. 1956. Temporal studies of cell division. I. The influence of ploidy and temperature on cell division in *S. cerevisiae*. J. Cellular Comp. Physiol. **47**:357–376.
5. CHUNG, C. W., AND W. J. NICKERSON. 1954. Polysaccharide syntheses in growing yeasts. J. Biol. Chem. **208**:395–407.
6. CIFONELLI, J. A., AND F. SMITH. 1955. The polysaccharide associated with yeast invertase. J. Am. Chem. Soc. **77**:5682–5684.
7. CROOK, E. M., AND I. R. JOHNSTON. 1962. The qualitative analysis of the cell walls of selected species of fungi. Biochem. J. **83**:325–331.
8. EDDY, A. A. 1958. The structure of the yeast cell wall. II. Degradative studies with enzymes. Proc. Roy. Soc. (London) Ser. B **149**:425–440.

9. FALCONE, G., AND W. J. NICKERSON. 1956. Cell-wall mannan-protein of baker's yeast. Science **124**:272–273.
10. FALCONE, G., AND W. J. NICKERSON. 1959. Enzymatic reactions involved in cellular division of microorganisms, p. 65–70. *In* W. J. Nickerson [ed.], Biochemistry of morphogenesis. Pergamon Press, London.
11. FLORY, P. J. 1956. Role of crystallization in polymers and proteins. Science **124**:53–60.
12. FREIFELDER, D. 1960. Bud position in *Saccharomyces cerevisiae*. J. Bacteriol. **80**:567–568.
13. FRENCH, C. S., AND H. W. MILNER. 1955. Disintegration of bacteria and small particles by high-pressure extrusion, p. 64–67. *In* S. P. Colowick and N. O. Kaplan [ed.], Methods in enzymology, vol. 1. Academic Press, Inc., New York.
14. FULLER, A. T. 1938. The formamide method for the extraction of polysaccharides from haemolytic Streptococci. Brit. J. Exptl. Pathol. **19**:130–139.
15. GARCIA MENDOZA, C., AND J. R. VILLANUEVA. 1962. Production of yeast protoplasts by an enzyme preparation of *Streptomyces* sp. Nature **195**:1326–1327.
16. GARZULY-JANKE, R. 1940. Über das Vorkommen von Mannan bei Hyphen- und Sprosspilzen. Zentr. Bakteriol. Parasitenk. Abt. II **102**:361–365.
17. HASENCLEVER, H. F., AND W. O. MITCHELL. 1960. Antigenic relationships of *Torulopsis glabrata* and seven species of the genus *Candida*. J. Bacteriol. **79**:677–681.
18. HATCH, M. D., AND J. F. TURNER. 1960. A protein disulphide reductase from pea seeds. Biochem. J. **76**:556–562.
19. HESTRIN, S. 1949. The reaction of acetylcholine and other carboxylic acid derivatives with hydroxylamine, and its analytical application. J. Biol. Chem. **180**:249–261.
20. HOUWINK, A. L., AND D. R. KREGER. 1953. Observations on the cell wall of yeasts: an electron microscope and X-ray diffraction study. Antonie van Leeuwenhoek J. Microbiol. Serol. **19**:1–24.
21. HOUWINK, A. L., D. R. KREGER, AND P. A. ROELOFSEN. 1951. Composition and structure of yeast cell walls. Nature **168**:693–694.
22. HUGHES, D. E. 1951. A press for disrupting bacteria and other microorganisms. Brit. J. Exptl. Pathol. **32**:97–109.
23. HURST, H. 1952. An electron diffraction study of the structure and orientation of the lipids in yeast and bacterial cell walls. J. Exptl. Biol. **29**:30–53.
24. JONSEN, J. 1955. Serological studies in fungi. Comparison of agglutinins and precipitins in anti-Candida sera. Acta Pathol. Microbiol. Scand. **37**:79–83.
25. JONSEN, J., S. RASCH, AND Å. STRAND. 1955. Specific polysaccharides in genus Candida. I. Preparation and properties of water soluble fractions. Acta Pathol. Microbiol. Scand. **37**:449–458.
26. KEMP, G. A. 1961. Antigenicity and pathogenicity of *Candida albicans* infections in mice. Ph.D. Thesis, Rutgers University, New Brunswick, N.J.
27. KESSLER, G., AND W. J. NICKERSON. 1959. Glucomannan-protein complexes from cell walls of yeasts. J. Biol. Chem. **234**:2281–2285.
28. KLAUSHOFER, H., H. MÄHR, AND A. SZILVINYI. 1961. Zellwandfraktionierungen an Sacch. carlsbergensis und Sacch. pastorianus. Mitt. Versuchssta. Gaerungsgewerbe Inst. Angew. Mikrobiol. **15**:1–8.
29. KORN, E. D., AND D. H. NORTHCOTE. 1960. Physical and chemical properties of polysaccharides and glycoproteins of the yeast cell wall. Biochem. J. **75**:12–17.
30. KREGER, D. R. 1954. Observations on cell walls of yeasts and some other fungi by X-ray diffraction and solubility tests. Biochim. Biophys. Acta **13**:1–9.
31. LAND, E. H. 1935. Method of making a product containing oriented asymmetric bodies. U. S. Patent 1,989,371.
32. LEE, Y. C., AND R. MONTGOMERY. 1962. Glycopeptodes from ovalbumin: the structure of the peptide chains. Arch. Biochem. Biophys. **97**:9–17.
33. MARTIN, D. S., C. P. JONES, K. F. YAO, AND L. E. LEE, JR. 1937. A practical classification of the monilias. J. Bacteriol. **34**:99–129.
34. MILLER, M. W., AND H. J. PHAFF. 1958. The cell wall composition of the apiculate yeasts. Antonie van Leeuwenhoek J. Microbiol. Serol. **24**:225–238.
35. MORRIS, E. O. 1958. Yeast growth, p. 251–321. *In* A. H. Cook [ed.], The chemistry and biology of yeasts. Academic Press, Inc., New York.
36. MORTIMER, R. K., AND J. R. JOHNSTON. 1959. Life span of individual yeast cells. Nature **183**:1751–1752.
37. NICKERSON, W. J. 1942. Mechanics of budding and of conjugation in yeasts. J. Cellular Comp. Physiol. **19**:379–382.
38. NICKERSON, W. J. 1954. An enzymatic locus participating in cellular division of a yeast. J. Gen. Physiol. **37**:483–494.
39. NICKERSON, W. J., AND C. W. CHUNG. 1954. Genetic block in the cellular division mecha-

nism of a morphological mutant of a yeast. Am. J. Botany **41**:114–120.

40. NICKERSON, W. J., AND G. FALCONE. 1956. Identification of protein disulfide reductase as a cellular division enzyme in yeasts. Science **124**:722–723.
41. NICKERSON, W. J., AND G. FALCONE. 1959. Function of protein disulfide reductase in cellular division of yeasts, p. 409–424. *In* R. Benesch [ed.], Sulfur in proteins. Academic Press, Inc., New York.
42. NICKERSON, W. J., G. FALCONE, AND G. KESSLER. 1961. Polysaccharide-protein complexes of yeast cell walls, p. 205–228. *In* M. V. Edds, Jr. [ed.], Macromolecular complexes. Ronald Press, New York.
43. NICKERSON, W. J., W. A. TABER, AND G. FALCONE. 1956. Physiological bases of morphogenesis in fungi. 5. Effect of selenite and tellurite on cellular division of yeast-like fungi. Can. J. Microbiol. **2**:575–584.
44. NORTHCOTE, D. H. 1953. The sorption of water vapour by yeast cell wall and other polysaccharides. Biochim. Biophys. Acta **11**:471–479.
45. NORTHCOTE, D. H., AND R. W. HORNE. 1952. The chemical composition and structure of the yeast cell wall. Biochem. J. **51**:232–236.
46. PECK, R. J. 1947. The lipids of fungi with special reference to pathogenic fungi, p. 167–188. *In* W. J. Nickerson [ed.], Biology of pathogenic fungi. Ronald Press, New York.
47. PHAFF, H. J., H. TANAKA, AND L. W. HIGGINS. 1961. Hydrolysis of yeast cell walls by bacterial enzymes. Bacteriol. Proc., p. 62.
48. REBERS, P. A., AND F. SMITH. 1954. The constitution of Iles mannan. J. Am. Chem. Soc. **76**:6097–6102.
49. ROBSON, J. E. 1958. Sulphur metabolism in fungi, p. 28–43. *In* Radioisotopes in scientific research, vol. 4. Pergamon Press, London.
50. ROBSON, J. E., AND H. M. STOCKLEY. 1962. Sulphydryl metabolism of fungi grown in submerged culture. J. Gen. Microbiol. **28**:57–68.
51. ROELOFSEN, P. A. 1953. Yeast mannan, a cell wall constituent of baker's yeast. Biochim. Biophys. Acta **10**:477–478.
52. ROMANO, A. H., AND W. J. NICKERSON. 1954. Cystine reductase of pea seeds and yeasts. J. Biol. Chem. **208**:409–416.
53. SALTON, M. R. J. 1955. Isolation of Streptomyces spp. capable of decomposing preparations of cell walls from various micro-organisms and a comparison of their lytic activities with those of certain actinomycetes and mycobacteria. J. Gen. Microbiol. **12**:25–30.
54. SENTHESHANMUGANATHAN, S., AND W. J. NICKERSON. 1962. Composition of cells and cell walls of triangular and ellipsoidal forms of *Trigonopsis variabilis*. J. Gen. Microbiol. **27**:451–464.
55. SHIFRINE, M., AND H. J. PHAFF. 1958. On the cell wall composition of *Saccharomycopsis guttulata*. Antonie van Leeuwenhoek J. Microbiol. Serol. **24**:274–280.
56. SKINNER, C. E., AND D. W. FLETCHER. 1960. A review of the genus *Candida*. Bacteriol. Rev. **24**:397–416.
57. SMITH, F., AND H. C. SRIVASTAVA. 1956. Acetolysis of the glucomannan of Iles mannan. J. Am. Chem. Soc. **78**:1404–1408.
58. STODOLA, F. H. 1958. Chemical transformations by microorganisms. John Wiley & Sons, Inc., New York.
59. SVIHLA, G., F. SCHLENK, AND J. L. DAINKO. 1961. Spheroplasts of the yeast *Candida utilis*. J. Bacteriol. **82**:808–814.
60. TREVELYAN, W. E., AND J. S. HARRISON. 1951. Studies on yeast metabolism. I. Fractionation and microdetermination of cell carbohydrates. Biochem. J. **50**:298–303.
61. TSUCHIYA, T., Y. FUKAZAWA, S. AMENIYA, M. YONEZAWA, AND K. SUZUKI. 1957. Serological classification of the genus Rhodotorula. Yokohama Med. Bull. **8**:215–224.
62. TSUCHIYA, T., Y. FUKAZAWA, L. SATO, S. KAWAKITA, M. YONEZAWA, AND Y. YAMASE. 1958. Serological classification of the genus Saccharomyces. Yokohama Med. Bull. **9**:359–370.
63. TSUCHIYA, T., K. KIYOAKI, F. YOSHIMURA, M. FUKUO, AND S. KAWAKITI. 1956. Studies on the classification of the genus *Candida*. V. Relation of the isolated strains to the new antigenic structure. Yokohama Med. Bull. **7**:127–132.
64. VON EULER, H., AND A. FODOR. 1911. Zur Kenntnis des Hefengummis. Z. Physiol. Chem. **72**:339–346.
65. WARD, J. M., AND W. J. NICKERSON. 1958. Respiratory metabolism of normal and divisionless strains of *Candida albicans*. J. Gen. Physiol. **41**:703–724.
66. WHISTLER, R. L., AND J. N. BEMILLER. 1958. Alkaline degradation of polysaccharides. Advan. Carbohydrate Chem. **13**:289–329.